

## Expression of the *Bacillus subtilis* *dinR* and *recA* Genes after DNA Damage and during Competence

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Received 30 October 1991/Accepted 7 March 1992

**The *Bacillus subtilis* *dinR* gene product is homologous to the LexA protein of *Escherichia coli* and regulates the expression of *dinR* and *dinC*. Using transcriptional fusions in the *dinR* and the *recA* genes, we have investigated the epistatic relationship between these two genes during the SOS response induced either by DNA damage or by competence. The results show that after DNA damage, induction of the expression of both *recA* and *dinR* is dependent on the activity of the DinR and RecA proteins. A RecA-dependent activity on DinR is proposed as the initial event in the induction of the SOS network. In contrast, the competence-related induction of *dinR* and *recA* appears to involve two distinct mechanisms. While one mechanism corresponds to the classical regulation of the SOS response, the other appears to involve an activating factor. Moreover, this factor is active in cells in which competence is prevented by a mutation in the regulatory gene *comA*.**

In *Bacillus subtilis*, an SOS system is induced when cells are exposed to conditions that damage DNA or undergo the competence state. Several phenomena that occur during the SOS response caused by DNA damage have been described elsewhere: overexpression of the *din* and *recA* genes (13), induction of DNA damage repair (12), increase in the rate of mutagenesis (26), Weigle reactivation of prophages (5), induction of the lytic cycle of prophages (24), induction of the SP $\beta$  DNA methylase (25), and development of cell filamentation (12). To date, three damage-inducible genes (*din*) have been identified: *dinA* (*uvrA*), *dinB*, and *dinC* (10, 11), as well as a *uvrB* gene (2, 18). Recently, a new *din* gene, *dinR*, was identified for *B. subtilis*. The *dinR1* mutant was found to be deficient in both recombination and DNA repair. The DinR protein shares significant amino acid sequence similarities (47.3%) with the LexA protein of *Escherichia coli* (19). LexA is the repressor of the SOS gene network, and like LexA, DinR appears to be a regulator of the expression of other *din* genes (19, 22). In *E. coli*, induction of the SOS system results from the reversible activation of RecA, leading to the cleavage of the repressor of the SOS genes (22). The amino acid sequences in the three regions known to be required for the cleavage of *E. coli* LexA are highly conserved in DinR, suggesting that DinR could undergo similar RecA-mediated cleavage. *E. coli* and *B. subtilis* RecA proteins have several regions of similarity (21), and *B. subtilis* RecA is immunoreactive with antibodies raised against *E. coli* RecA. Furthermore, the *B. subtilis* RecA protein can facilitate *E. coli* LexA cleavage in vitro (14), and the *E. coli* RecA protein expressed on a plasmid in *B. subtilis* complements, at least partially, the *recA4* (previously *recE4*) mutant for recombination, induction of *din* genes, and Weigle reactivation (13, 16). However, the *E. coli* RecA protein is unable to restore prophage induction in the *recA4* mutant of *B. subtilis* (13), and the *B. subtilis* RecA cannot promote the cleavage of the  $\lambda$ CI repressor molecule in vitro (14).

An additional regulation of the SOS system occurs when *B. subtilis* cells develop competence, a physiological state in

which the cells are able to bind and take up exogenous DNA. Competence is expressed after exponential growth and is subject to three types of regulation: nutritional, growth-stage specific, and cell-type specific (for a review, see reference 4). Several genes that regulate the development of competence have been identified. The *comA* and *comP* genes belong to the family of two-component regulators (23) and encode a response regulator and a histidine kinase protein, respectively. The regulatory *com* genes are expressed throughout the *B. subtilis* growth cycle. To express late competence genes in addition to the identified early *com* gene products, a limiting transcription factor is necessary. A DNA fragment upstream of the promoter region of the *comDE*, *comC*, and *comG* genes appears to bind this competence transcription factor (CTF) (4, 17). The SOS system appears to be partially derepressed during competence, as judged by overexpression of the *din* (including *dinR*) and *recA* genes (11, 15, 19). The induction of the *dinA*, *dinB*, and *dinC* genes is observed only in the presence of wild-type RecA activity. During competence, the increased synthesis of RecA does not require the presence of a functional RecA protein; in the *recA4* mutant, the competence-related stimulation of *recA* expression is observed, whereas overexpression of *recA* following DNA damage is inhibited (15). Overexpression of *dinR* is also observed during competence, even in the presence of the mutant DinR1 protein (19). These results indicate that another factor might be involved in the specific induction of the SOS response.

Within the promoter regions of several *din* genes, there are conserved sequences that have been proposed as recognition elements for the binding of a presumably common SOS-specific regulatory protein. These sequences have also been detected upstream of the *recA*, *recM*, and *dinR* genes (3, 19). In addition, a sequence that resembles the putative CTF binding element was found upstream of the *recA* gene (4). This finding raised the possibility that, in addition to the classical SOS regulation, a dual regulation of *recA* involved the binding of CTF and the subsequent displacement of the repressor of the SOS genes, leading to induction of *recA* expression during competence (4).

We have investigated the epistatic relationship between *dinR* and *recA* by measuring (i) expression of *recA* in

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TABLE 1. *B. subtilis* strains used in this study

| Strains                 | Genotype and/or relevant characteristics   | Reference or source |
|-------------------------|--|---------------------|
| 1680 <sup>+</sup>       | Wild type, prototrophic  | Our collection      |
| MO506                   | <i>trpF7 ura-1 thr-5 acf-1 rfm-486</i>   | Our collection      |
| MO534                   | <i>dinR1::Tn917lac</i>   | 19                  |
| MO556                   | 1680 <sup>+</sup> [pB16] <sup>a</sup> ; Campbell recombinant <sup>b</sup> in <i>dinR</i> | 19                  |
| BD1626                  | <i>hisA1 metB leu comA124::pTV21Δ2</i> ; Lac <sup>-</sup> derivative                     | 9                   |
| YB886/ <i>recA::cat</i> | <i>metB5 trpC2 xin-1 SPβ<sup>-</sup> recA2 (recA::cat)</i>                               | R. Yasbin           |
| QB4444                  | <i>trpC2 recA2 (recA::aphA3)</i>   | F. Kunst            |
| BG225                   | <i>metB5 trpC2 xin-1 SPβ<sup>-</sup> (recA::xylE)</i>                                    | J. Alonso           |
| MO551                   | <i>comA124::pTV21Δ2</i>  | This work           |
| MO569                   | <i>dinR1::Tn917lac</i>   | This work           |
| MO579                   | <i>dinR1::Tn917lac recA2 (recA::cat)</i>   | This work           |
| MO572                   | 1680 <sup>+</sup> [pB16] <i>recA2 (recA::aphA3)</i>                                      | This work           |
| MO573                   | 1680 <sup>+</sup> [ <i>recA::xylE</i> ]  | This work           |
| MO574                   | <i>dinR1::Tn917lac [recA::xylE]</i>  | This work           |

<sup>a</sup> Brackets indicate an integrated plasmid molecule.

<sup>b</sup> Campbell recombinant refers to a strain that was made by transformation with a circular DNA molecule.

wild-type, merodiploid, and mutant *dinR* backgrounds and (ii) expression of *dinR* in both wild-type and *recA* backgrounds. These experiments were conducted after induction of the SOS response either by DNA-damaging agents or when cells developed competence. The results presented here indicate that, after DNA damage, the expression of *recA* and *dinR* is dependent on the wild-type activity of both DinR and RecA proteins. When cells become competent, in addition to this regulation, an unidentified factor seems to be necessary for the expression of *dinR* and *recA*.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and growth media.** *B. subtilis* strains were derivatives of wild-type strain 168 and are listed in Table 1. Construction of the *dinR* merodiploid strain that contains both the *dinR-lacZ* fusion and a wild-type copy of *dinR* has been previously reported (19). The *recA::cat* mutation from the strain YB886-*recA::cat* was transduced into the *dinR1* strain MO534 to create the strain MO569. The strain MO556 was constructed by Campbell-like recombination between the pB16 plasmid, which carries the *dinR-lacZ* fusion and the *cat* gene, and the chromosome of the wild-type strain. Because strain MO556 was resistant to chloramphenicol, we performed its transformation with a *recA::aphA3* null allele (Km<sup>r</sup>; recently constructed by F. Kuntz). The resulting strain, MO579, contains the *dinR1-lacZ* fusion along with an intact copy of the *dinR* gene and the *recA::aphA3* mutation. Chromosomal DNA from QB4444, a strain which carries the *recA::aphA3* mutation, was used to transform competent MO556 cells to kanamycin resistance (Km<sup>r</sup>), thereby generating the strain MO579. We renamed *recA::cat* and *recA::aphA3* the *recA2* allele of *recA*; in these constructions, the *cat* or *aphA3* gene was inserted into the *Clal* site of *recA*. To introduce the *recA::xylE* fusion into the wild-type and *dinR1* strains, we used the strain BG225, which carries the *recA::xylE* gene fusion ectopically inte-

grated onto the chromosome. The chromosomal DNA purified from BG225 (*recA::xylE*) was mixed (1:1) with DNA purified from MO506 (Rif<sup>r</sup>); Rif<sup>r</sup> *recA::xylE* recombinants were then obtained by congression of these two markers into the wild-type strain and strain MO556. The Rif<sup>r</sup> recombinants were selected in rich medium in the presence of rifamycin and then sprayed with a 0.5 M solution of catechol to reveal the activity of the *xylE* gene product, catechol 2,3-oxygenase (catO<sub>2</sub>ase) (27). By a similar method, the wild-type strain was also transformed for *dinR::Tn917lac* and *recA::xylE*; in this case, DNA from BG225 (*recA::xylE*) was mixed with DNA purified from MO534 (*dinR1::Tn917lac* Ery<sup>r</sup>), and recombinants were selected on medium containing erythromycin and sprayed with the catechol solution. The strains MO572 (*recA<sup>+</sup> recA::xylE rfm-486*), MO573 (*dinR::Tn917lac recA<sup>+</sup> recA::xylE*), and MO574 (*dinR<sup>+</sup> dinR::Tn917lac recA<sup>+</sup> recA::xylE rfm-486*) were subsequently purified by streaking them on rich medium in the presence of the appropriate antibiotic. The *comA124* mutation from BD1626 (9) was moved into the strain MO534 by transformation.

Plasmid pB16 has been previously described (19) and contains the *Bgl*III promoter proximal clone of the *dinR::lacZ* fusion from strain MO542. Erythromycin at 5 µg/ml, lincomycin at 20 µg/ml, mitomycin (MC) at 50 or 150 ng/ml, rifamycin at 5 µg/ml, kanamycin at 5 µg/ml, chloramphenicol at 5 µg/ml, and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) at 40 µg/ml were utilized.

**Genetic and molecular procedures.** Genetic transformation, transduction, development of competence, and chromosomal DNA purification were performed as described previously (6).

**Enzymatic assays.** β-Galactosidase assays were conducted by using bacteria grown in Luria-Bertani or Schaeffer's medium; no significant difference in the β-galactosidase activity between bacteria grown in each of the two media was observed. For the catO<sub>2</sub>ase assay, strains were grown in Luria-Bertani medium because the fusion has no activity in Schaeffer's medium. For measuring enzymatic activities during competence, a one-step protocol was used as described previously (1). In particular experiments, MC was added at 150 ng/ml during the exponential phase of growth. The level of β-galactosidase activity was determined as described previously (8). β-Galactosidase specific activities are expressed as units per milligram of protein. The amount of protein was determined from standard curves by relating the turbidity (optical density at 570 nm) to the protein concentration of the bacterial cultures. Preparation of samples from culture and determination of catO<sub>2</sub>ase activity were performed as described elsewhere (27), with minor modifications: the cells were washed in 20 mM potassium phosphate buffer (pH 7.2) and resuspended in AP buffer (100 mM potassium phosphate buffer [pH 7.5] and 10% acetone [vol/vol]). Whole cells were disrupted by 30 min of incubation with 50 µg of lysozyme per ml at 37°C and then stored at 4°C. The catO<sub>2</sub>ase activity was determined by following the increase in A<sub>375</sub> due to the accumulation of 2-hydroxy-muconic semialdehyde. Milliunits of activity are defined as described by Sala-Trepas and Evans (20).

## RESULTS

**Expression of *dinR* after DNA damage.** The previously characterized *dinR1* mutant was deficient in both recombination and DNA repair, and the expression of the *dinR1* allele was noninducible by DNA damage. In the *dinR*

TABLE 2. Effect of RecA and DinR on the expression of the *dinR-lacZ* transcriptional fusions following DNA damage

| Time after induction (h) | $\beta$ -Galactosidase sp act (Units/mg of protein) <sup>a</sup> |     |                              |     |   |     |   |     |
|--------------------------|--|-----|------------------------------|-----|---|-----|---|-----|
|                          | MO534 ( <i>dinR1 recA</i> <sup>+</sup> )                         |     | MO569 ( <i>dinR1 recA2</i> ) |     | MO556 ( <i>dinR</i> <sup>+</sup> / <i>dinR1 recA</i> <sup>+</sup> ) |     | MO579 ( <i>dinR</i> <sup>+</sup> / <i>dinR1 recA2</i> ) |     |
|                          | –MC  | +MC | –MC                          | +MC | –MC   | +MC | –MC   | +MC |
| –0.5                     | 43   |     | 20                           |     | 135   |     | 56  |     |
| 0                        | 25   |     | 16                           |     | 113   |     | 59  |     |
| 0.5                      | 28   | 33  | 32                           | 30  | 120   | 160 | 71  | 59  |
| 1                        | 50   | 45  | 33                           | 30  | 163   | 295 | 92  | 74  |
| 2                        | 68   | 66  | 46                           | 35  | 163   | 530 | 79  | 47  |
| 3                        | 64   | 65  | 60                           | 25  | 161   | 722 | 52  | 44  |

<sup>a</sup> –MC, growth in rich medium without added MC; +MC, growth in rich medium with added MC (150 ng/ml).

merodiploid strain MO556, which carries an intact copy of the *dinR* gene coexistent with the *dinR1::Tn917lac* mutation, recombination and DNA repair capacities were restored, and the expression of the *dinR* gene became inducible by DNA-damaging agents (19). To investigate the role of RecA on the expression of *dinR*, a *recA2* null allele (*recA::cat* or *recA::aphA3*) was introduced into strains MO534 (*dinR1*) and MO556 (*dinR*<sup>+</sup>/*dinR1*), each of which contains a *dinR-lacZ* fusion. The double mutant strain MO569 (*dinR1 recA2*) had a lower growth rate than the parental *dinR1* strain, which had a rate of growth equivalent to that of the wild-type strain. The doubling time of strain MO569 in rich medium was 35 min, whereas the doubling time of strain MO534 was 23 min. In addition, strain MO569 demonstrated a phenotype of extensive filamentation, which was not observed in the *recA* or *dinR* mutants.

The  $\beta$ -galactosidase activities of cultures of MO534 (*dinR1 recA*<sup>+</sup>), MO569 (*dinR1 recA2*), MO556 (*dinR*<sup>+</sup>/*dinR1 recA*<sup>+</sup>), and MO579 (*dinR*<sup>+</sup>/*dinR1 recA2*) were measured, in the presence or absence of MC (Table 2). Increased expression of the *dinR* gene in the presence of MC was seen only in strain MO556 (*dinR*<sup>+</sup>/*dinR1 recA*<sup>+</sup>);  $\beta$ -galactosidase activity observed 3 h after the addition of MC was 4.5-fold greater than that observed in the absence of MC. In addition, the basal level of *dinR-lacZ* expression in this strain was three- to fourfold higher than that of the other strains. In contrast, no significant increase in the expression of the *dinR-lacZ* fusion in the presence versus the absence of MC was observed in strains MO534 (*dinR1 recA*<sup>+</sup>), MO569 (*dinR1 recA2*), and MO579 (*dinR*<sup>+</sup>/*dinR1 recA2*). Comparison of *dinR* expression in strain MO556 (*dinR*<sup>+</sup>/*dinR1 recA*<sup>+</sup>) with its expression in strain MO579 (*dinR*<sup>+</sup>/*dinR1 recA2*) indicates that the absence of a functional RecA protein prevents the induction of the *dinR* gene after DNA damage. These results suggest that a RecA-dependent activity is necessary for the induction of *dinR* expression following DNA damage.

**Effect of DinR on *recA* expression following MC treatment.** The DinR protein is a protein that regulates the expression of both *dinR* and *dinC* (19). Another important protein in the function of the SOS response in *B. subtilis* is RecA. To measure the effect of DinR on the transcription of the *recA* gene, a *recA::xylE* gene fusion was introduced into the wild-type, *dinR1*, and merodiploid *dinR*<sup>+</sup>/*dinR1* strains. All of the resultant strains are merodiploid for the *recA* gene, with the wild-type copy of *recA* at its normal position on the *B. subtilis* chromosome and the *recA::xylE* fusion integrated ectopically. Expression of the *recA::xylE* fusion was measured in the following strains: MO572 (wild type), MO573 (*dinR1*), and MO574 (*dinR*<sup>+</sup>/*dinR1*) (Table 3). The levels of *recA* expression during growth were extremely different for

the three strains. When the *recA::xylE* fusion was introduced into the *dinR1* mutant strain (MO573), the basal level of *recA* expression was only 10% of that of the wild-type strain (MO572). In the *dinR* merodiploid strain (MO574), the level of *recA::xylE* expression was approximately twofold higher than that of the fusion in the wild-type strain. In all of these strains, a 7- to 10-fold increase of catO<sub>2</sub>ase activity was observed when cultures in rich medium entered the stationary phase of growth. A similar unexplained increase was observed by Gassel and Alonso when the *recA::xylE* fusion was carried on a multicopy plasmid (7).

In the wild-type strain grown in rich medium, expression of the *recA::xylE* fusion started to increase 1 h after the addition of MC. After 3 h of MC treatment, the *recA::xylE* expression increased to 5.5-fold its expression in the untreated culture (Table 3 and Fig. 1). Induction of *recA* expression was also observed in the *dinR* merodiploid background (strain MO574), indicating that wild-type regulation of *recA* expression occurs in the merodiploid strain. In contrast, no induction of the *recA::xylE* fusion occurred in strain MO573 after MC treatment. These results suggest the following hypotheses. (i) DinR is the repressor for the damage-inducible genes and is inactivated after DNA damage, thereby leading to the expression of the SOS genes. In this case, the DinR1 protein is a noninactivable mutant protein which acts as a super repressor. (ii) DinR is a positive regulator necessary for the induction of *recA*. Furthermore, the low level of expression of *recA* found in the *dinR1* mutant might explain the Rec<sup>–</sup> phenotype of this strain (19).

**Effect of RecA on competence-induced *dinR* expression.** When *B. subtilis* cells reach competence, specific induction of the *din* and *recA* genes occurs (11, 15). While induction of

TABLE 3. Effect of DinR on the expression of the *recA::xylE* gene fusions following DNA damage

| Time after induction (min) | CatO <sub>2</sub> ase sp act (mU/mg of protein) <sup>a</sup> |      |                        |     |   |      |
|----------------------------|--|------|------------------------|-----|---|------|
|                            | MO572 ( <i>dinR</i> <sup>+</sup> )                           |      | MO573 ( <i>dinR1</i> ) |     | MO574 ( <i>dinR</i> <sup>+</sup> / <i>dinR1</i> ) |      |
|                            | –MC  | +MC  | –MC                    | +MC | –MC   | +MC  |
| 0                          | 1.2  |      | 0.1                    |     | 3.5   |      |
| 30                         | 1.0  | 1.4  | 0.2                    | 0.2 | 1.9   | 2.7  |
| 60                         | 2.1  | 6.0  | 0.5                    | 0.7 | 5.2   | 11.3 |
| 120                        | 3.1  | 16.7 | 1                      | 1.3 | 15.1  | 37.0 |
| 180                        | 7.4  | 40.6 | 1.5                    | 1.0 | 23.3  | 97.5 |

<sup>a</sup> –MC, growth in rich medium without added MC; +MC, growth with added MC (150 ng/ml).

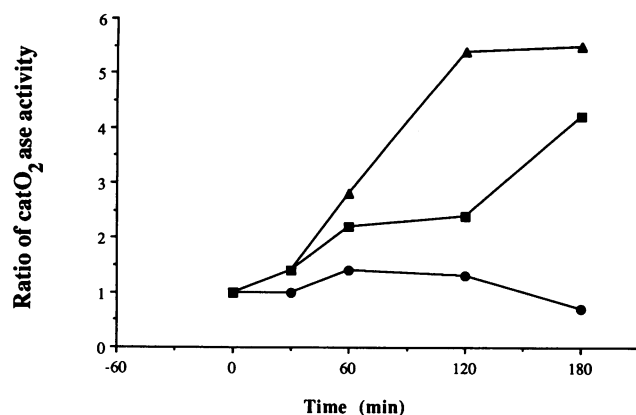


FIG. 1. Expression of *recA::xylE* following DNA damage. The ratios of catO<sub>2</sub>ase activity between MC-treated and untreated cultures are presented. MC (150 ng/ml) was added in vegetative growth (optical density at 600 nm of 0.3 to 0.5). Time is expressed in minutes, with zero representing the time of MC addition. ▲, MO572 (wild type); ●, MO573 (*dinR1*); ■, MO574 (*dinR*<sup>+</sup>/*dinR1*).

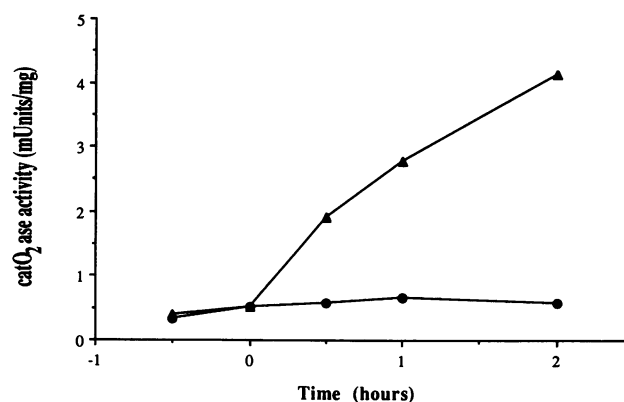


FIG. 2. Expression of *recA::xylE* during the development of competence. Strains MO572 (wild type) and MO573 (*dinR1*) were grown according to a one-step competence protocol, and catO<sub>2</sub>ase activity was assayed at the indicated times. Time is expressed in hours, with zero (*T*<sub>0</sub>) representing the end of exponential growth. ▲, MO572; ●, MO573.

the *dinA*, *dinB*, and *dinC* genes is dependent on the wild-type RecA protein, induction of the *recA* gene occurs also in the presence of the RecA4 inactive protein. To investigate whether competence-related induction of *dinR* is dependent on the *recA* gene product, expression of the *dinR-lacZ* fusion in strains MO534 (*dinR1 recA*<sup>+</sup>), MO569 (*dinR1 recA2*), MO556 (*dinR*<sup>+</sup>/*dinR1 recA*<sup>+</sup>), and MO579 (*dinR*<sup>+</sup>/*dinR1 recA2*) was measured (Table 4). In the *dinR* merodiploid strain MO556, a threefold increase in *dinR* expression was observed when the cells developed competence. In strain MO579 (*dinR*<sup>+</sup>/*dinR1 recA2*), a twofold increase of *dinR* expression was observed, but only 2 h after the end of exponential growth. In addition, the basal level of *dinR* expression (at 0.5 h before end of log-phase growth; *T*<sub>-0.5</sub>) was fivefold lower in strain MO579 than in strain MO556. These results indicate that the RecA protein is (i) necessary for the normal timing of induction of *dinR* expression during competence and (ii) necessary for the maintenance of a basal level of DinR during growth.

A competence-related increase of β-galactosidase activity was also observed in strains MO534 (*dinR1*) and MO569 (*dinR1 recA2*) (Table 4), indicating that in these strains the augmentation of *dinR* expression occurs in the presence of the DinR1 protein and in the absence of RecA. These results may indicate that the competence-related *dinR* induction occurs independently of any activity of RecA on DinR. In addition, *dinR* expression during vegetative growth (*T*<sub>-0.5</sub>) was lower in both strains MO534 and MO569 than in strain MO556. This suggests that the DinR protein also contributes

to the basal level of *dinR* expression. Such a contribution is evident when strains MO556 and MO534 are compared.

**Effect of DinR on the competence-related induction of *recA*.** Previous work has shown that the *recA* gene is induced in competent cells (15). This induction occurs in the *recA4* mutant strain, which encodes an SOS-inactive form of the RecA protein. To investigate whether the competence-related induction of *recA* is dependent on the activity of DinR, the expression of the *recA::xylE* fusion was measured during competence. In the *dinR*<sup>+</sup> strain (MO572), the *recA::xylE* fusion was induced 10-fold 2 h after the end of exponential growth, whereas no induction of *recA* in the *dinR1* mutant was observed (Fig. 2). These results suggest that the competence-related overexpression of *recA* requires the presence of a wild-type DinR protein. Taking into account that only 10 to 20% of cells reach competence (4), the observed values (in terms of milliunits of catO<sub>2</sub>ase per milligram of protein) for competence-related *recA* induction are underestimates in these experiments.

**Effect of *comA* on competence-related expression of *dinR*.** Transcriptional activation of the *dinR* gene has been observed when *B. subtilis* becomes competent, even in the absence of any known DNA-damaging agent (19). The competence-related induction of *dinR* expression occurs in the *dinR1* mutant, thereby indicating that it is independent of the DinR wild-type protein. The induction of *dinR* could thus be controlled by *comA*, a major regulator of competence gene expression (9). To investigate the role of *comA* in competence-induced expression of *dinR*, the *comA124* allele was introduced by transformation into strain MO534 (see Materials and Methods). β-Galactosidase activities in the *dinR1* mutant (MO534) and in the *dinR1-comA* double mutant (MO551) were measured (Fig. 3). Comparable levels of expression of the *dinR* gene occurred in the two strains when the cells became competent, thereby indicating that *dinR* overexpression is independent of the *comA* gene product.

TABLE 4. Effect of RecA on the expression of the *dinR-lacZ* fusion during competence

| Strain | Relevant genotype   | β-Galactosidase sp act (U/mg of protein) <sup>a</sup> |                       |                       |                       |                       |
|--------|---|---|-----------------------|-----------------------|-----------------------|-----------------------|
|        |   | <i>T</i> <sub>-0.5</sub>                              | <i>T</i> <sub>0</sub> | <i>T</i> <sub>1</sub> | <i>T</i> <sub>2</sub> | <i>T</i> <sub>3</sub> |
| MO556  | <i>dinR</i> <sup>+</sup> / <i>dinR1 recA</i> <sup>+</sup> | 216   | 256                   | 420                   | 552                   | 654                   |
| MO534  | <i>dinR1 recA</i> <sup>+</sup>                            | 82  | 84                    | 167                   | 204                   | 246                   |
| MO569  | <i>dinR1 recA2</i>  | 51  | 46                    | 92                    | 169                   | 247                   |
| MO579  | <i>dinR</i> <sup>+</sup> / <i>dinR1 recA2</i>             | 44  | 46                    | 55                    | 92                    | 101                   |

<sup>a</sup> *T*<sub>0</sub>, the end of the exponential growth (subscript numerals are in hours).

## DISCUSSION

The development of the competence state in *B. subtilis* is accompanied by induction of the *din* and *recA* genes, the expression of which is also induced by DNA-damaging agents (11, 15). Both the competence- and the DNA damage-

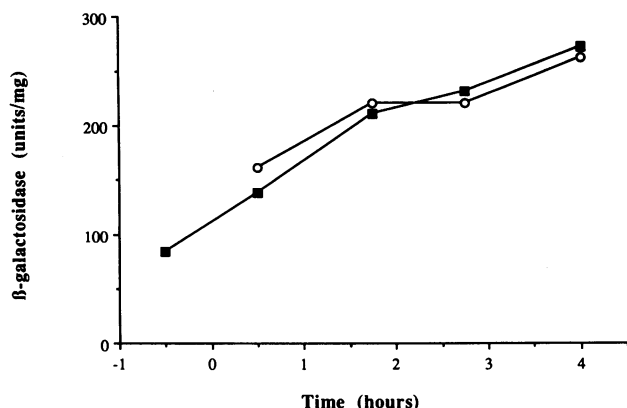


FIG. 3. Expression of *dinR*::Tn917lac fusion during the development of competence. Strains MO551 (*dinR1 comA124*) and MO534 (*dinR1*) were grown according to a one-step competence protocol, and  $\beta$ -galactosidase activity was assayed at the indicated times. Time is expressed in hours, with zero ( $T_0$ ) representing the end of exponential growth.  $\circ$ , MO551;  $\blacksquare$ , MO534.

related inductions of the *din* genes require a functional RecA protein. In contrast, while the induction of *recA* expression after DNA damage requires a functional RecA protein, the competence-induced expression of *recA* occurs independently of the presence of active RecA (15). The *recA* gene seems to be under two types of regulation, one active after DNA damage and the other active when the cells reach competence.

We have previously reported the identification of *dinR*, a regulator of *din* gene expression, which encodes a protein homologous to the LexA protein of *E. coli* (19). The *dinR1* mutant, originally identified as deficient for homologous recombination, encodes a truncated form of the DinR protein which lacks 20% of the wild-type protein at the carboxy terminus. Expression of *dinR* is induced following DNA damage and when the cells become competent. In contrast to the DNA damage induction of *dinR*, its competence-related induction occurs in the *dinR1* mutant. In this work, we have investigated the epistatic relationship between the *B. subtilis* *recA* and *dinR* genes during both the SOS response and the development of competence.

Induction of a *dinR-lacZ* fusion following DNA damage was abolished in both *recA2* and *dinR1* mutants, indicating that similar effects result from the presence of the DinR1 protein (or absence of DinR) and from the absence of the active RecA protein. These observations support two hypotheses. (i) The amino acid motifs known to be necessary for the cleavage of the *E. coli* LexA protein are conserved in DinR, suggesting that the DinR protein could undergo a similar RecA-mediated cleavage (19). In this case, DinR normally acts as a repressor of the SOS genes and the DinR1 protein is a noncleavable form of DinR. (ii) DinR is a positive effector necessary for the expression of the SOS genes, and the DinR1 protein has lost this activity (discussed below).

Expression of the *recA* gene is highly influenced by the *dinR* allele present in the cell. In the presence of the *dinR1* mutation, *recA* expression was only 10% of that obtained in the presence of the *dinR* wild-type allele. This low rate of *recA* expression in the *dinR1* background might explain the  $\text{Rec}^-$  phenotype of the DinR1 mutant (19). In the *dinR* merodiploid strain, both DinR and DinR1 are synthesized, and the level of *recA::xylE* expression was approximately

twofold higher than that of the fusion in the wild-type strain. Thus, our results show that *dinR*, *dinC*, and *recA* are poorly expressed in the presence of the *dinR1* allele and that in the merodiploid strain the level of expression of *recA* is higher than it is in the wild-type strain. These results lead us to favor the first hypothesis, in which DinR acts as a repressor for damage-inducible genes such as *recA*, *dinC*, and *dinR*. The presence of the conserved SOS boxes in the regulatory regions of these genes and of *dinA*, *dinB*, and *recM* (3, 19) suggests a possible interaction of DinR with these DNA sequences. From the results concerning *dinR* and *recA* expression during vegetative growth, we can conclude that a reciprocal regulatory effect allows a basal level of expression of these genes. A permanent low level of DinR expression appears to be necessary to ensure a basal quantity of RecA, which accounts for the induction of the SOS response. The presence of a DinR1 protein leads to an SOS induction-deficient phenotype. In contrast, RecA wild-type activity is needed to maintain the DinR constitutive level and to derepress *dinR* transcription following DNA damage. These results allow a comparison between the SOS responses of *B. subtilis* and *E. coli* (22), in which there is an interdependence of RecA and LexA in the regulation of the SOS network.

*dinR* expression, like that of the *din* and *recA* genes, is induced when the cells become competent. In this case, the RecA protein has no effect on *dinR* induction in the *dinR1* mutant, supporting the hypothesis that the DinR1 protein is insensitive to RecA activity. Moreover, the absence of the RecA protein in the merodiploid strain was associated with a diminution of competence-related *dinR* induction. These results suggest that the DinR protein synthesized in the merodiploid strain can be modified by some RecA activity, leading to overexpression of the *dinR* gene. This mechanism is consistent with dependence on RecA for the induction of the other *din* genes during competence. Moreover, in the *dinR1* strain, when the cells reached competence, induction of *recA* expression was abolished whereas that of *dinR1* was still present. These results suggest that DinR1 regulation of the *recA* promoter prevents *recA* induction. However, the activity of RecA on DinR does not seem to be the only way to activate *dinR* expression during competence. In the *dinR1-recA2* double mutant, as in the *dinR1* strain, the competence-related overexpression of *dinR* is still observed. Thus, the RecA-dependent activity on DinR, necessary for SOS induction, could be bypassed by another mechanism during competence. The competence-related overexpression of *recA* depends on the presence of a DinR wild-type protein (Fig. 2); nevertheless, this induction is also observed in the *recA4* mutant, which encodes an inactive form of the RecA protein (14). These results could also indicate that the RecA-dependent activity on DinR is not necessary for the induction of the *recA* gene; it is possible that the *dinR* and *recA* genes are also under the regulation of an unidentified activator that is independent of the SOS regulation. This putative factor would be able to displace DinR (or DinR1) from its target sequence without RecA-dependent activity, thereby permitting the transcription of the *dinR* or the *recA* gene. Overexpression of *dinR* during competence was observed in the *dinR1 comA* double mutant, indicating that the competence-induced expression of *dinR* is independent of the regulator ComA. Thus, expression of the putative activator of *dinR* must also be independent of ComA. This putative factor is different from the CTF evoked as a regulator of the SOS competence phenomenon, since CTF is not synthesized in the *comA124* mutant (4).

In conclusion, we propose the following hypothesis to

explain the double regulation of *dinR* and *recA* expression observed during competence. When competence is induced in the wild-type cell, a low level of expression of the *dinR* and *recA* genes occurs by a mechanism that involves the presence of an activating factor that displaces DinR from the *dinR* and *recA* promoters. This mechanism does not involve any activity of RecA on the DinR protein, thereby explaining the overproduction of DinR and RecA during competence in the *dinR1* and *recA4* mutants. A competition between DinR1 and the putative positive activating factor for binding to the *recA* promoter would account for the low level of transcription of *recA* in the *dinR1* mutant and for its Rec<sup>-</sup> phenotype. In parallel, the SOS signal might be produced by a limited rate of DNA replication in competent cells (4). At this time, the survival of the cell would be guaranteed by a derepression of the SOS functions which might be due to RecA-mediated cleavage of DinR. Subsequently, full induction of the SOS system occurs, and the resultant increased quantity of RecA allows genetic recombination and derepression of the *din* genes.

#### ACKNOWLEDGMENTS

We thank J. Alonso, F. Kunst, and R. Yasbin for providing strains YB886/*recA::cat*, QB4444, and BG225. We also thank R. D'Ari, M. Goldberg, C. Parsot, P. Sansonetti, and A. Zychlinsky for helpful discussions.

A.R.D. is a fellow of the Ligue Nationale Française Contre le Cancer.

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